REMARKS

By the present communication, claims 1-4, 26, 32 and 38 have been amended. No new matter is introduced by the subject amendments as the amended claim language is fully supported by the specification and original claims.

Upon entry of the amendments submitted herewith, claims 1-44 remain pending, with claims 1-7, 21, 24-26 and 32-38 under active consideration, and claims 8-20, 22, 23, 27-31 and 39-44 withdrawn from consideration, subject to a request for rejoinder thereof. A detailed listing of claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented herein, beginning on page 2 of the present communication, with an appropriate status identifier.

Claim Rejections· 35 USC § 101

The rejection of claims 1-7, 21, 24-26 and 32-38 under 35 USC § 101 as allegedly being directed to non-statutory subject matter is respectfully traversed. It is respectfully submitted that this rejection is not applicable to the amended set of claims provided herewith. Accordingly, reconsideration and withdrawal of the rejection under 35 USC § 101 are respectfully requested.

Claim Rejections - 35 USC § 102

The rejection of claims 1, 32-35, 37 and 38 under 35 USC § 102(e)(2) as allegedly being anticipated by Hilser et al. (U.S. Pat. No. 7,027,969) is respectfully traversed. Applicants' invention, as defined for example by claim 1, distinguishes over Hilser et al. at least by requiring a method of three-dimensional structure determination of a protein of interest of unknown structure. Invention method comprises:

(i) experimentally determining the protein amide hydrogen exchange rate profile for each of the several peptide amide hydrogens in the protein employing peptide amide hydrogen-deuterium exchange mass spectrometry,

(ii) predicting a set of possible structures for the protein,

(iii) calculating the peptide amide hydrogen exchange rate profile for each predicted possible structure in the set,

(iv) comparing calculated rates of amide hydrogen exchange determined for each member of the set of predicted possible structures for said protein of interest with the experimentally determined hydrogen exchange rate profile of said protein of interest, and

(v) identifying one or more structures for said protein from said set of predicted possible structures having a calculated exchange rate profile closely matching the experimental exchange rate profile for said protein.

Thus, the instant application is directed to the determination of the structures of proteins of unknown structure. All calculations of hydrogen exchange rates are performed on the speculative 3-D coordinates of the members of a set of hypothesized, predicted structures for the protein of unknown true structure.

The protein structure prediction art is presently inexact; therefore, it is reasonably expected that most members of a set of hypothesized structures for an unknown protein will not accurately represent the protein's (unknown) true structure. Thus, in the instant method, the large majority of exchange rate calculations are performed on structural predictions within the set that are inaccurate predictions, such that the rare accurate predictions in the set are discerned only by separate reference to the experimentally determined (DXMS analysis) rate information for the protein. The invention method never seeks to calculate hydrogen exchange rates using the coordinates of a protein of known true structure. In contrast, Hilser et al. teaches the calculation of hydrogen exchange rates only of proteins of known structure.

Indeed, Hilser et al. do not teach or suggest any utility of comparison of calculated hydrogen exchange rates with DXMS-experimentally determined hydrogen exchange rates. Instead, Hilser et al. teach algorithmic-based computational predicting, calculating, and determining of amide hydrogen exchange rates and other thermodynamic parameters of proteins by use of the COREX algorithm. However, Hilser et al. only contemplate that said predicting,

calculating, and determining is to be done by applying COREX and like algorithms to the 3-D structural coordinates of a protein who's actual high-resolution (true) structure has been determined *a priori* by experimental methods including x-ray crystallography and NMR. There is no teaching or suggestion of the use or utility of application of COREX calculations to the hypothesized 3-D coordinates for speculative proposed models for a protein of unknown true structure. Hilser et al. exclusively teach the application of their invention to proteins of known structure, and all of their examples and proposed applications rely on the *a priori* availability of a high-resolution experimentally determined 3-D structure for the protein to be computationally studied. Each of the six examples presented in Hilser et al. employ COREX calculations performed on the 3-D coordinates of the previously determined high resolution crystallographically-derived true structure of the protein SNase (1stn.pdb). To quote Hilser et al.:

> The crystallographic structure of SNase (1stn.pdb) was used as a template to computationally generate an ensemble of partially folded states using the COREX algorithm...

(see the first line of Example 2). Hilser et al. does not describe or contemplate performing such calculations on proteins of unknown structure.

The Examiner's efforts to bolster the rejection based on Hilser et al. by quoting language out of context from the reference is without merit. Specifically, Applicants disagree with the Examiner's assertion that Hilser et al. allegedly

> . . . further teaches embodiments wherein the above described computational procedure [COREX] is used characterize hypothetical structure of ensembles and identifying and designing real world proteins that exhibit the predicted characteristics of said calculated ensembles (see Hilser et al. col. 10, line 35 through col. 12, line 30).

(See page 6, lines 6-10 of the Office Action). Inspection of the indicated section of the Hilser et al. specification (entitled "II. Protein Design", see col. 10, line 33), demonstrates that it is directed to use of computational techniques in the design of new proteins and protein-binding entities. All examples and descriptions in Hilser et al. of proposed methods to this end begin

with the application of computational techniques to previously-determined high resolution structures for the target protein to be "re-designed". To quote directly from Hilser et al.,

> Thus in specific embodiments, the present invention comprises a method of designing a protein pharmaceutical exhibiting increased stability comprising the steps of inputting a high resolution structure of a protein: generating an ensemble of incrementally different conformational states by combinatorial unfolding of a set of pre-defined folding units in all possible combinations of the protein: determining the probability of each of said conformational state...

(see Hilser et al. col. 10, lines 41-48; emphasis added). In addition, Hilser et al. assert that

> Another embodiment is a method of designing an oral protein pharmaceutical exhibiting increased adsorption in the gastrointestinal tract comprising the steps of inputting high resolution structure of a protein: generating an ensemble of incrementally different conformational states by combinatorial unfolding of a set of pre-defined folding units in all possible combinations of the protein: determining the probability of each of said conformational state...

(see Hilser et al. col. 10, lines 55-62; emphasis added). And

> . . . in one approach, one would generate a three-dimensional structure for the protein or a fragment thereof by X-ray crystallography...

(Hilser et al. col. 11, lines 30-33). And

> It is also possible to isolate a protein specific antibody. Selected by a functional assay, and then solve its crystal structure.

(Hilser et al. col. 11, lines 27-29). Furthermore, the "ensembles" of structures referred to in this section of Hilser et al. are not sets of structural predictions for structurally folded proteins, but are specifically noted to be various unfolded (non-native) representations of a single protein's previously determined high resolution structure.

These unfolded representations are not novel, proposed structures for a protein (for example as part of protein design) upon which COREX is then applied, but instead are the

ensemble of unfolded target protein representations that are at the heart of the functioning of the COREX algorithm for any use, and are generated by the intrinsic functioning of the COREX algorithm itself. Consistent with the preceding discussion, note that in the Brief Summary of the Invention, Hilser et al. assert that

> In the present invention, the COREX algorithm is used to generate an ensemble of partially folded states based on the crystallographic structure of a protein...An embodiment of the present invention is a method of calculating the microscopic pKa of a protein comprising the steps of: inputting a high resolution stricture of the protein: generating an ensemble of incrementally different conformational states by conformational unfolding...

(Hilser et al. col. 1, line 60-col. 2, line 9). The above cited section of Hilser et al. is devoid of any mention of the use of COREX to calculate the hydrogen exchange behavior of any protein, unfolded, folded, or otherwise in the protein design methods presented. In contrast, the instant invention specifically employs computational methods to calculate the hydrogen exchange behavior of hypothesized protein structures, and makes use of no other calculated values for the protein.

Taken together, this evidence indicates that the instant method is novel in the use of computational methods to calculate the hydrogen exchange rates of structural predictions for proteins (not unfolded proteins) that have unknown structures, as specified in Claims 1 and 38. Accordingly, reconsideration and withdrawal of the rejection under 35 USC § 102(e)(2) are respectfully requested.

Claim Rejections - 35 USC § 103

The rejection of claims 1 and 32-38 under 35 USC § 103(a) as allegedly being unpatentable over Hilser et al., in view of Simons et al. (PROTEINS: Structure, Function, and Genetics, Supplement, vol. 3, pages 171-176 (1999)) is respectfully traversed. As noted above,

Hilser et al. do not disclose or suggest the present invention as claimed herein. Further reliance on Simons et al. is unable to cure the deficiencies of Hilser et al.

For example, in one embodiment, the instant invention employs the COREX algorithm to calculate the hydrogen exchange rates of amide hydrogens (hydrogen exchange rate profile) for each member of a set of structures (folded structures) hypothesized for a protein of unknown structure. In this embodiment, Rosetta is used to generate each member of the desired set of structural predictions. With the input of the protein's primary amino acid sequence, Rosetta does this by variously folding (not unfolding) progressive segments of the protein's known primary amino acid sequence (Simons et al., 1999). Thus Rosetta progressively determines and assigns the structural coordinates of each element of the protein, and when finished with the calculation, the result is a possible complete folded structure for the protein. This process is repeated many times by Rosetta in a manner whereby varied possible folded structures for the protein are calculated for the same input primary amino acid sequence.

In accordance with the present invention, each member of this set of predictions is then independently subjected to the action of the COREX algorithm, which proceeds by a process, presented in detail in Hilser et al. (US Patent No. 7,029,969), that begins by progressive unfolding or "disappearing" portions of a structure. Thus the purposes, actions, and results of the Rosetta *vs* COREX algorithms are entirely different and distinct: COREX, as presented in Hilser et al., relies on computational unfolding (disordering) of progressive portions of a protein of known structure, while Rosetta relies on the progressive assembly of an entirely disordered primary amino acid sequence into a highly folded, possible 3-D structure for the protein.

In contrast to the uses described in the prior art, according to the instant invention, Rosetta and COREX are combined in a novel and non-obvious manner, with each algorithm having an entirely distinct function: While Rosetta is employed to predict many possible structures, COREX is used to calculate, for each structure, its hydrogen exchange rate profile. Unfortunately, however, this information, by itself, provides no assistance in the identification of

which particular Rosetta-calculated structure is an accurate representation of the true structure. It is only in accordance with the instant method that one is taught (1) to compare each predicted exchange rate profile with the "true" exchange rate profile experimentally measured for the protein by DXMS analysis, and this comparison is then used (2) to identify predicted structures in the set that have COREX-generated profiles that match it, and thereby identify the accurate structural prediction.

To summarize there are at least two substantial differences between the instant invention and the prior art, i.e.,:

1. Computational methods such as COREX are used to calculate hydrogen exchange rate profiles for hypothesized (not actual) protein structures, and

2. The experimentally determined hydrogen exchange rate profile of a protein, measured by DXMS analysis, is used to identify structural predictions that have matching COREX-calculated exchange rate profiles, thereby identifying those structural predictions which are accurate.

By themselves, items 1 and 2 have no practical or obvious utility. There is no obvious utility in performing COREX calculations on virtual 3-D structures, and stopping there; similarly, there is no obvious utility in performing COREX rate determination on a structurally determined protein and comparing it to DXMS data; nor is there any obvious method of determining unknown protein 3-D structures by DXMS experimental analysis alone. Only the present invention contemplates the combined use of COREX with DXMS analysis in such a way that is feasible, practical, and potentially of great utility.

Accordingly, reconsideration and withdrawal of the rejection under 35 USC § 103(a) over the combination of Hilser et al. in view of Simons et al. are respectfully requested.

The rejection of claims 1-7, 21, 24-26, 32-35, 37 and 38 under 35 USC § 103(a) as allegedly being unpatentable over Hilser et al., is respectfully traversed. For at least the reasons set forth above, it is respectfully submitted that Hilser et al. do not disclose or suggest the present invention as claimed herein.

Accordingly, reconsideration and withdrawal of the rejection under 35 USC § 103(a) over Hilser et al. are respectfully requested.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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